

## A New Species of Nototheniid (Perciformes: Notothenioidei) Fish from McMurdo Sound, Antarctica

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A new species of nototheniid fish, *Cryothernia amphitreta*, is described from a single gravid female collected in mid-November 2004 by divers in McMurdo Sound in the Ross Sea region of Antarctica. The new species closely resembles the only known congener, *C. peninsulae*, collected off the west coast of the Antarctic Peninsula, but differs substantially in pelvic-fin length (13.4 vs. 19.3–24.4% SL), total vertebrae (57 vs. 50–53), body size at maturity (261 vs. 100–144 mm), and interorbital-pit morphology. The neutrally-buoyant *C. amphitreta* is characterized by a wide, well-defined interorbital pit divided by a raised medial ridge, scales anterior to this depression in the interorbital region, and a dark pigmentation of the mouth, gill, and body cavity linings. This species is protected against freezing by high levels of antifreeze proteins in its body fluids. Phylogenetic reconstruction using the mitochondrial NADH dehydrogenase subunit 2 (mtND2) suggests that *C. amphitreta* falls within the current designation of the nototheniid subfamily Trematominae.

IN mid-November 2004, divers conducting routine surveys at a heavily-sampled site in McMurdo Sound, Ross Sea, Antarctica happened upon a fish of unknown identity. A single gravid female was collected, resembling the nototheniid *Cryothernia peninsulae*, which has been collected only near the tip of the Antarctic Peninsula (Daniels, 1981; DeWitt et al., 1990). Investigations revealed several distinct differences between *C. peninsulae* and the unidentified specimen, which is described here as *Cryothernia amphitreta*, representing the second species of the genus and the first *Cryothernia* to be collected in the Ross Sea region.

Previous morphological examinations of *Cryothernia peninsulae*, the type species for this genus, have led to the placement of *Cryothernia* within the nototheniid subfamily Pleuragramminae (Andersen, 1984; DeWitt et al., 1990) with *Pleuragramma* and *Aethotaxis*. This conclusion was based primarily on the elements of the cephalic lateral-line canal pores and the placement of the pectoral foramen within the scapula. However, also citing features of the laterosensory system, Balushkin (1984, 2000) placed *Cryothernia* within the subfamily Trematominae along with the genera *Pagothenia* and *Trematomus*, as designated by DeWitt et al. (1990). Buoyancy measurements by Eastman (1985) also led to the conclusion that *C. peninsulae* is more similar to the Trematominae than to *Pleuragramma* or *Aethotaxis*. Although recovery of DNA from *C. peninsulae* is not possible due to the use of a formalin-based fixative for all known specimens (Daniels, 1981), phylogenetic studies using DNA sequences obtained from the holotype of *C. amphitreta* may help to resolve questions concerning the evolu-

tionary relationships of both species of *Cryothernia* to other species within the nototheniid radiation.

The accessibility of ice-free land, open water, and annual sea ice in the western Ross Sea, Antarctica has allowed investigations of the fish fauna for over a century, despite its extreme southern latitude. Nevertheless, even well-sampled areas of the Southern Ocean continue to yield new species of fishes (Eakin and Eastman, 1998), and it seems that McMurdo Sound is no exception.

### MATERIALS AND METHODS

A single specimen of unknown identity was returned to the McMurdo Station aquarium and held in a tank with other fishes for approximately one week for observation, buoyancy measurements, and photographs. The fish was anesthetized with 0.2% (w/v) tricaine methanesulfonate (MS-222, Sigma Chemical Co., St. Louis, MO) and sacrificed, tissues were sampled and frozen, and the fish was preserved in a 10% formalin solution. Prior to preservation, the visceral organs were removed and frozen for later DNA and RNA analyses, but a portion of the intestine, the pyloric caeca, and a few eggs were left intact and preserved along with the fish. The stomach was empty.

To determine the antifreeze protein content of the serum, blood was obtained from the caudal vein of the anesthetized fish with a 23-G needle and 3-ml syringe. The blood was allowed to clot at 4 C for several hours and the serum was recovered after centrifugation (10,000 × g). Native and heated (10 min, 100 C) serum freezing points (FP) were determined using the capillary

method as described by DeVries (1971, 1986) and Jin and DeVries (2006). The serum melting point (MP, in C) was calculated from the osmotic concentration (OC, in Osm) determined with a Vapro 5520 vapor pressure osmometer (Wescor Inc., Logan, UT) using  $-1.858 \times OC = MP$ . Serum thermal hysteresis (TH) was calculated using  $TH = MP - FP$ .

Measurements and counts, excluding those of the cephalic lateral line, were taken according to Hubbs and Lagler (1958). Pectoral- and pelvic-fin lengths were measured from the extreme base of the uppermost or anteriormost ray to the farthest tip of the fin. All measurements were taken with digital measuring calipers to the nearest 0.1 mm and are reported as % SL unless otherwise noted. Fin-ray and other counts were carried out under a stereomicroscope at appropriate magnifications. The cephalic lateral-line pore counts and examinations follow Illick (1956). Film radiographs were obtained for vertebrae counts and pectoral and caudal skeleton morphology using standard veterinary equipment, scanned into a computer, and examined using digital imaging software. The description of the caudal skeleton follows Andersen (1984). Due to the loss of scales while in the aquarium, only general observations of lateral-line morphology are presented here; pored-scale counts were estimated by counting lateral-scale series in the vicinity of the lateral line. The weight of the live, anesthetized fish was measured in air and local seawater according to Eastman and DeVries (1982), and observations of egg buoyancy were taken from re-hydrated (3 hrs in standard seawater at 4 C) formalin-fixed eggs.

DNA was obtained from previously frozen ( $-80$  C) liver tissue by phenol-chloroform extraction and ethanol precipitation (Sambrook and Russell, 2001) for use in polymerase chain reaction (PCR) amplification and direct DNA sequencing of the mitochondrial NADH dehydrogenase subunit 2 (mtND2) gene. PCR and sequencing of the 1047-nucleotide (nt) mtND2 gene used the primers described by Cziko et al. (2006), with PCR reaction conditions described by Cheng et al. (2003). Direct sequencing of the amplified genes was accomplished using BigDye v3.0 (Applied Biosystems Inc., Foster City, CA) chemistry with the manufacturer's recommended reaction conditions and an ABI 3730xl capillary sequencer.

Phylogenetic analysis of the entire mtND2 gene sequences from the holotype of *C. amphitreta* and seven other members of the Nototheniidae was used to gain insight into the phylogenetic relationships of *C. amphitreta* within the Nototheniidae. Representatives of six of the 13 genera from the nototheniid subfamilies (DeWitt

et al., 1990) Nototheniinae, Pleuragramminae, and Trematominae were included in the analysis. The basal nototheniid *Dissostichus mawsoni* was used as the outgroup (Balushkin, 2000). Maximum-likelihood (ML) phylogenetic tree reconstruction was implemented in PAUP (vers. 4.0b10, D. L. Swofford, PAUP\*: phylogenetic analysis using parsimony [\*and other methods], Sinauer, Sunderland, MA, 2003) using the heuristic search option and a sequence evolution model (GTR + I +  $\Gamma$ ) for nucleotide substitution rates and invariant sites, and a  $\Gamma$ -distribution for among-site rate variation (J. Nylander, 2004, MrModeltest vers. 2.2, program distributed by the author, Uppsala University, Sweden; Waddell and Steel, 1997; Posada and Crandall, 1998). Maximum parsimony (MP) tree reconstruction was implemented in Mega v3.1 (Kumar et al., 2004) using the max-mini branch-and-bound routine with all nucleotides equally weighted for 2000 bootstrap pseudoreplicates.

#### *Cryothernia amphitreta*, new species

Figures 1, 2A

*Holotype*.—USNM 385901, female, 261 mm SL, 405 g (303 mm fresh TL; weight 1.8 g in seawater), Antarctica, Ross Sea, eastern McMurdo Sound, McMurdo Station saltwater intake jetty,  $77^{\circ}51.033'S$ ,  $166^{\circ}39.759'E$ , collected using a hand net at 20 m under approx. 5 m sea ice, 15 Nov. 2004.

*Diagnosis*.—A species of *Cryothernia* with an outline similar to that of *C. peninsulæ* but with pelvic fins reaching only about halfway from pelvic-fin base to anal origin, and second dorsal-fin insertion notably advanced with respect to the anal-fin origin (Fig. 1). Cephalic lateral-line pores large, the fourth supraorbital canal pores open into a median, well-defined, depressed interorbital pit, divided medially by a slightly raised ridge (Fig. 2A). Width of depressed interorbital region greater than the length, does not extend anteriorly beyond the third supraorbital canal pore, and with a few uniserial cycloid scales immediately anterior to it. Lining of the mouth, gill cavities, and peritoneum coal-black.

*Description*.—Gravid female, 261 mm SL: Body fusiform and slightly compressed caudally, head depressed. Maximum body depth at origin of second dorsal fin 25.6; width at this point 14.6; depth at anal origin 22.7. Preanal length 52.2; predorsal length 29.9. Mouth large, the lower jaw projecting slightly. Head length 27.4; depth at occiput 18.3; width at vertical edge of preopercles

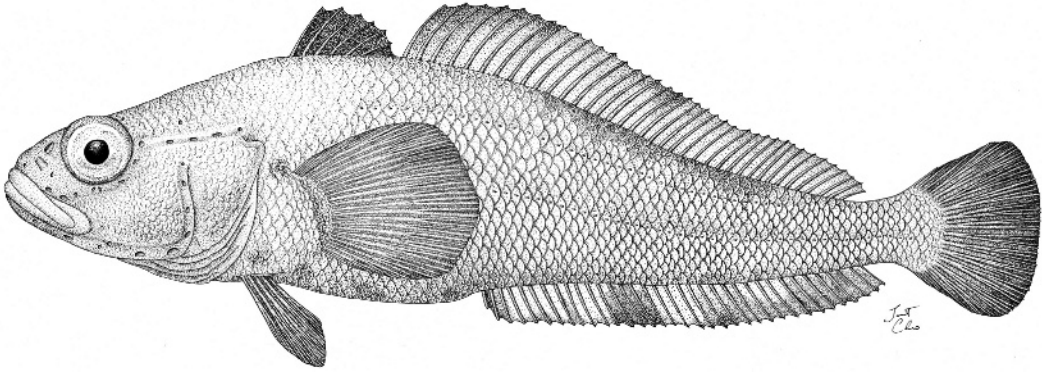


Fig. 1. *Cryothenia amphitreta*, holotype, USNM 385901, gravid female, 261 mm SL, collected by divers at approx. 20 m depth in McMurdo Sound, Antarctica (77°51.033'S, 166°39.759'E).

17.0; cheek length 14.6. Eyes lateral, but with the ventralmost margin of the orbit at the midline. Orbit diameter 8.3, notably larger than both bony 4.7, and fleshy 5.8, interorbital widths. Postorbital head length 12.8. Lower jaw projects slightly and extends to vertical at middle of eye; upper-jaw length 29.5; jaw width 10.8 at posterior ends of maxillaries. Jaws with small conical teeth in two to three rows anteriorly, becoming uniserial posteriorly. Upper and lower pharyngeal teeth present in typical percomorph pattern; teeth absent from vomer and palatines. Snout blunt and short 7.7; nostrils tubular; internostril distance 6.3; tip of snout to nostril 4.5; nostril to orbit 1.9; nostril diameter 1.0. Cephalic sensory canal pores moderately large but not prominent, no long canaliculi associated with the canal pores. Mandibular canals each with four pores, not uniting at symphysis; first and second small and circular, third larger and elliptical. Preopercular canals not connected to mandibular or temporal segments; preopercular canal with seven pores, numbers 2–5 slit-like. Infraorbital canal with eight small pores beginning ventral to nostril; infraorbital canal divided between pores 5 and 6, just below the eye. Supraorbital canals with five pores, beginning anterior to the nostril and extending caudally to temporal canal; first pore small, second large; the fourth supraorbital canal pore opens into a wide, well-defined pit divided medially by a slightly raised ridge (Fig. 2). Temporal canal with five pores, branching at the third pore into a very short supratemporal canal, ending in a single pore; no supratemporal commissure. Arrangement of cephalic sensory canals identical to *C. peninsulae* (DeWitt et al., 1990) excepting the division in the infraorbital canal, which was also seen in approximately half of the specimens examined by Daniels (1981). Maximum width of depressed

interorbital region 2.6; length 1.5; length at midline 1.2. Four gill arches, pseudobranch well developed. Gill rakers short, roughly conical, non-dentigerous; lateral gill rakers on first arch (right side, upper + lower = total)  $7 + 16 = 23$ ; longest lateral gill raker 1.1, near arch angle; medial gill rakers on first arch  $1 + 17 = 18$ . Six branchiostegal rays. No elongate fin rays or spines. Pelvic fins relatively short 13.4, reaching about half of the minimum pelvic-fin base to base of first anal-fin ray distance, 25.7; five branched pelvic rays with a slender spine closely attached to the first branched ray; base length 3.5. Pectoral fins broad-based 10.0, large, but not overly long 22.5; reach just to vertical at first anal-fin ray; 25 and 26 (L and R) branched rays. Minimum pectoral base to pectoral base distance 10.4; minimum pectoral base to pelvic base distance 8.0, minimum pectoral base to first anal-fin ray base distance 20.3. First dorsal fin with six short, non-pungent spines; base length 7.4; first spine length 5.1, second spine longest, 5.6. Interdorsal distance (from base of last spine to base of first ray) 3.3; base length of second dorsal fin 53.1; 38 second dorsal-fin rays, a few branching at tips. Base of anal-fin length 44.3; 35 rays, some branching at tips; first anal-ray length 4.0, second 4.8, anteriormost anal-fin rays with blunt ends. Caudal fin 13.7 from middle of base to tip of longest ray, rounded with 13 branched rays; distance from posteriormost dorsal soft-ray base to caudal fin 7.8; from anteriormost anal-fin ray 27.4. Caudal-peduncle length 6.4; width 2.3; depth 6.2. Body scales ctenoid; head scales cycloid. Opercle, occiput, and most of cheek covered with scales; snout, preorbital, and lower jaw without scales; interorbit with a few scales (discussed above). Lateral lines with pored scales, no tubular scales present. Upper lateral line with approx. 35 pored scales; lower with approx. 39;

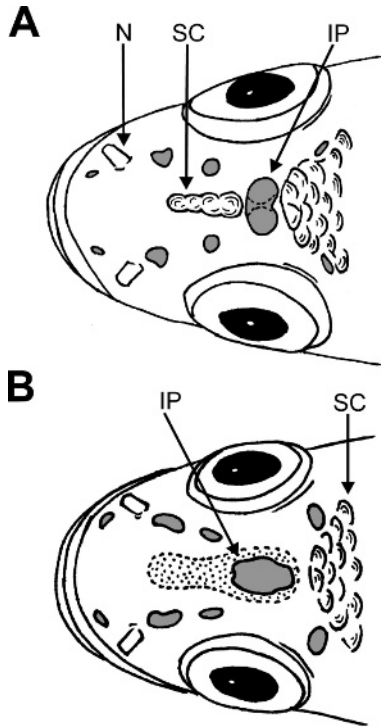


Fig. 2. Arrangement of the pores (shaded grey) of the supraorbital sensory canals, interorbital pit, and head scales in (A) *Cryothenia amphitreta*, holotype 261 mm SL and (B) *Cryothenia peninsulae*, based on NYSM 11457 paratype 112 mm SL. For *C. peninsulae*, the interorbital pit is surrounded by a depressed area (stippled). Figures are not drawn to the same scale. N = nostril, SC = scales, IP = interorbital pit.

due to scale damage definitive pored-scale counts not possible. Upper lateral line follows dorsal outline from below fourth dorsal-fin spine (no canal or pored scales were evident between this point and the temporal canal) to below about the thirty-second dorsal-fin ray (sixth from ultimate ray); middle lateral line follows midline from caudal fin to above origin of anal fin; no lower lateral line present. Vertebrae (abdominal + caudal = total)  $20 + 37 = 57$ ; pectoral foramen entirely within scapula; caudal skeleton appears to be of the  $2 + (2) + 1$  type (fusion of first and second hypurals, partial fusion of third and fourth, fifth hypural autogenous; examined from radiographs only). Seven pyloric caeca of roughly equal length 4.6 (mean), and diameter 1.0 (mean at midpoint). Ovaries ripe with many large (approx. 4 mm diameter) negatively buoyant eggs (re-hydrated formalin-fixed, alcohol-preserved) as well as many small (approx. 1 mm diameter) eggs for a subsequent season's spawn.

*Coloration of holotype.*—In life, body nearly uniformly bronze-colored with silver undertones and a notable iridescence; becoming uniformly silver over a few days in a lighted aquarium. A few indistinct darker blotches present dorsally and caudally, and ventral to the pectoral fin. Spinous dorsal fin blackish; caudal fin and pectoral fins uniformly dark; other fins becoming blackish distally, anal fin with irregular dark blotches. Lining of the mouth, gill cavities, and peritoneum coal-black. In alcohol (70% isopropyl, approx. one year) body pale pink with pinpoint dark chromatophores throughout. Body cavities and caudal fin retain blackish pigmentation; other fins, including pectoral fin, grayish and lighter than in life. In preserved specimen, the black lining of gill cavities extends past the opercular margin.

*Blood antifreeze protein content.*—*Cryothenia amphitreta* native serum, capillary method (DeVries, 1971) 1.11 C thermal hysteresis (TH). Heated serum (10 min, 100 C) 0.74 C TH; heating the serum allows for an estimation of the TH contribution due to antifreeze glycoprotein (AFGP) only, by denaturing the heat-labile antifreeze potentiating protein (AFPP; Jin and DeVries, 2006).

*Remarks and comparison.*—*Cryothenia amphitreta* (Fig. 1) is the second known species of the genus and the first to be discovered in the Ross Sea region of Antarctica. *Cryothenia amphitreta* does not exhibit the barred caudal and pectoral-fin coloration typical of *C. peninsulae*, and many of the cephalic lateral-line pores are much less prominent in *C. amphitreta* due to a reduction in their relative sizes. Other notable differences between these species of *Cryothenia* include the degree of pigmentation of body cavities, fin patterning and coloration, and differences in the relative positions of the dorsal- and anal-fin origins. For *C. peninsulae*, the holotype and largest collected specimen (144 mm SL) was a gravid female, and Daniels (1981) noted that "other specimens > 110 mm SL were approaching reproductive condition." Because notothenioids generally attain between 70–80% (some 55%, and rarely as low as 40%) of their maximum size before becoming reproductively mature (Kock and Kellermann, 1991), *C. amphitreta* (261 mm SL) is probably a larger species with a maximum SL of at least twice that of *C. peninsulae*. The divided infraorbital canal (between pores 5 and 6) observed in *C. amphitreta* was also found in about half of the 20 specimens of *C. peninsulae* (Daniels, 1981). Counts and

TABLE 1. MEASUREMENTS (% STANDARD LENGTH) AND COUNTS FOR *Cryothernia amphitrete* AND *C. peninsulae*.

Character	<i>C. amphitrete</i> (holotype) USNM 385901	<i>C. peninsulae</i> mean value <sup>a</sup>	<i>C. peninsulae</i> range of values <sup>a</sup>
Standard length (mm)	261.1	100	54.2–144
Head length	27.4	29.3	27.9–31.2
Head depth at posttemporals	21.8	19.2 <sup>b</sup>	16.8–21.5 <sup>b</sup>
Head width at preopercles	17.0	16.9 <sup>b</sup>	15.3–18.3 <sup>b</sup>
Orbit diameter	8.3	8.3	6.9–9.8
Bony interorbital width	4.7	5.3 <sup>b</sup>	5.0–5.7 <sup>b</sup>
Fleshy interorbital width	5.8	6.2 <sup>b</sup>	5.5–6.9 <sup>b</sup>
Snout length	7.7	7.8	7.3–8.9
Internostril distance	6.3	6.9 <sup>b</sup>	6.6–7.1 <sup>b</sup>
Upper-jaw length	11.3	12.2	11.5–13.0
Jaw width	10.8	12.9 <sup>b</sup>	10.9–15.5 <sup>b</sup>
Postorbital length of head	12.8	16.9 <sup>b</sup>	15.6–18.0 <sup>b</sup>
Body depth at second dorsal-fin origin	25.5	20.4 <sup>b</sup>	17.7–22 <sup>b</sup>
Length of second dorsal-fin base	53.1	51.1 <sup>b</sup>	50.9–56.0 <sup>b</sup>
Length of anal-fin base	44.3	51.4 <sup>b</sup>	48.7–52.9 <sup>b</sup>
Length of pectoral fin	22.5	24.5	22.5–26.2
Length of pelvic fin	13.4	21.6	19.3–24.4
Max. length of interorbital depression <sup>c</sup>	1.5	6.8 <sup>b</sup>	6.4–7.6 <sup>b</sup>
Width of interorbital depression <sup>c</sup>	2.6	2.5 <sup>b</sup>	2.0–3.1 <sup>b</sup>
Length of interorbital depression at dorsal midline <sup>c</sup>	1.2	6.8 <sup>b</sup>	6.4–7.6 <sup>b</sup>
Length of longest lateral gill raker	1.1	1.9 <sup>b</sup>	1.8–2.2 <sup>b</sup>
Predorsal length	29.9	30.7	29.2–32.3
Preanal length	52.2	46.9	44.4–48.4
Tip of pelvic fin to anus distance	13.5	0.6 <sup>b</sup>	0.0–2.4 <sup>b</sup>
Anal-fin rays	35	33.9	33–35
Branched caudal-fin rays	13	12 (16.0) <sup>d</sup>	12 (14–17) <sup>d</sup>
Pelvic-fin rays	5	5	5
Pectoral-fin rays (L and R)	25 and 26	24.8	24–26
Upper lateral gill rakers on first arch	7	8.0	7–9
Lower lateral gill rakers on first arch	16	15.8	15–17
Scales (pores) in upper lateral line	35 <sup>e</sup>	34.5	30–42
Scales (pores) in middle lateral line	38 <sup>e</sup>	35.8	34–38
Abdominal vertebrae	20	16.5	13–18
Caudal vertebrae	37	34.8	33–37
Total vertebrae	57	51.4	50–53
Spines in first dorsal fin	6	5.3	4–6
Rays in second dorsal fin	38	34.9	34–36
Branchiostegal rays	6	6	6

<sup>a</sup> Values from Daniels (1981), holotype plus 19 paratypes, unless otherwise noted.

<sup>b</sup> Values from four paratypes examined by P. A. Cziko (NYSM 11457, 78.5 and 123.9 mm SL; LACM 38386.001, 76.5 and 123.9 mm SL).

<sup>c</sup> For *C. peninsulae*, this includes the depressed area surrounding the pit (stippled area, Fig. 2B).

<sup>d</sup> Values from DeWitt et al. (1990); mean unspecified count from Daniels (1981) in parentheses.

<sup>e</sup> Pored lateral-line scale counts are estimated, definitive counts not possible due to missing scales.

measurements of the two new species of *Cryothernia* are compared in Table 1.

As a species, *C. amphitrete* fits well within the diagnosis of *Cryothernia* given by Daniels (1981) and DeWitt et al. (1990). *Cryothernia* is characterized by single-pored (no tubes) scales in the upper and middle lateral lines, absence of a lower lateral line, a distinctive pit-like interorbital depression arising from the fourth supraorbital cephalic lateral-line pores, preopercular canals unconnected to the mandibular and temporal canals, and 22 to 26 short, non-dentigerous gill rakers with the longest of about 1–2% SL. The

genus differs from *Pleuragramma*, which has three visible lateral lines, from *Aethotaxis*, which has tubular lateral-line scale pores and numerous elongate gill rakers, and from *Gvozdarus*, which has tubular lateral-line scale pores and several canine-like teeth. *Cryothernia* also differs from the genus *Trematomus*, which has a complete (or divided into two to three segments) supratemporal canal and tubed lateral-line scales, and from *Pagothenia*, which has a “supratemporal canal divided into two short segments and a short branch from each temporal canal” (DeWitt et al., 1990).

The fusiform, compressed body, the black peritoneal, mouth and gill cavity linings, and a (gravid) weight in local seawater of less than 0.5% of its weight in air for *C. amphitrete* suggest at least a semipelagic or benthopelagic life-history; the pelagic *Pleurogramma antarcticum* also weighs approximately 0.5% in seawater, whereas benthic nototheniids (*Trematomus* spp.), and even the cryopelagic *Pagothenia borchgrevinki*, range from about 2–4% of their weight in air (Eastman and DeVries, 1981, 1982; Eastman, 1985). Based on buoyancy measurements of rehydrated, eviscerated, alcohol-preserved specimens, Eastman (1993) suggested that *C. peninsulae*, with a mean percentage weight in seawater of 4.17% ( $\pm 0.478\%$  SEM,  $n = 13$ ; Eastman, 1985), is more similar to the Trematominae than to the Pleurogramminae. However, because the live, anesthetized, and fully gravid (with negatively buoyant eggs) *C. amphitrete* was found to be nearly neutrally buoyant (0.5% its weight in air), it is possible that live *C. peninsulae* are also nearly neutrally buoyant, with the viscera providing significant static lift.

Like the benthic nototheniids (Eastman, 1993), when motionless *C. amphitrete* remains propped up on its pelvic fins and its ventral surface does not touch the substrate. Additionally, the thickened skin covering the relatively short pelvic fins and the anterior half of the anal fin of *C. amphitrete* are probable substrate contact specializations (Eastman and DeVries, 1982); thickened skin on these fins is less pronounced in *C. peninsulae* (pers. obs.). In the aquarium, *C. amphitrete* was observed to employ labriform locomotion under normal unstressed conditions, and like *Trematomus loennbergii* (Eastman, 1993; pers. obs.), when resting on the bottom nearly continuously fanned the pectoral fins. Although untested for *C. peninsulae*, the high antifreeze protein level in *C. amphitrete* serum is typical of the Antarctic notothenioids (DeVries, 1988; Jin and DeVries, 2006), and this species is therefore well suited for life in the freezing, icy waters of the high Antarctic.

**Phylogenetic studies.**—Phylogenetic reconstruction using the complete mtND2 gene sequence from *C. amphitrete* and other notothenioids (Figs. 3A, B) suggests that *C. amphitrete* is closely related to members of the genera *Pagothenia* and *Trematomus* as recognized by DeWitt et al. (1990). Although the analysis presented here is not exhaustive, both maximum-likelihood (ML) and maximum parsimony (MP) phylogenetic tree reconstructions placed *C. amphitrete* within the nototheniid subfamily Trematominae with strong MP bootstrap support and with a long branch

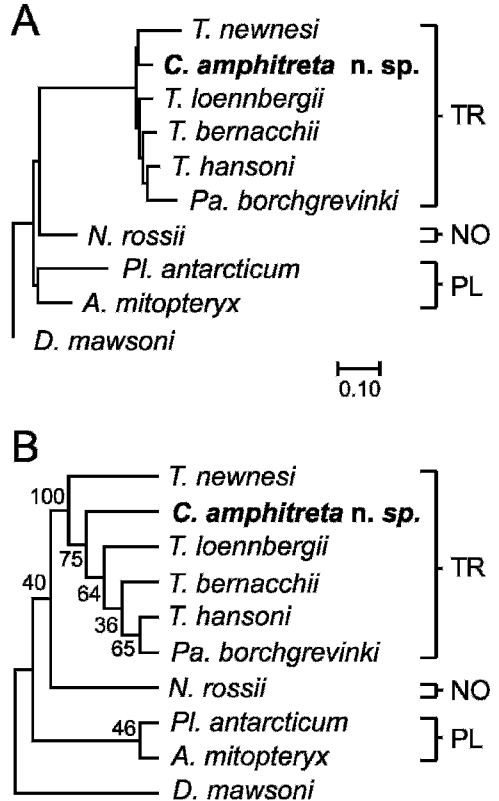


Fig. 3. Phylogenetic analysis of mtND2 gene sequence used to gain insight into the evolutionary relationships between *Cryothenia* and other members of the Nototheniidae. (A) Phylogram of the maximum-likelihood tree reconstruction; scale bar indicates 10% sequence divergence. (B) Cladogram of the maximum parsimony consensus tree with bootstrap values from 2000 pseudoreplicates. The tree topology within the Trematominae is not particularly well supported by the data. The basal nototheniid *D. mawsoni* was used as the outgroup. A = *Aethotaxis*, D = *Dissostichus*, N = *Notothenia*, Pa = *Pagothenia*, Pl = *Pleurogramma*, T = *Trematomus*; subfamilies in brackets, TR = Trematominae, NO = Nototheniinae, PL = Pleurogramminae.

length (ML, large sequence divergence) separating *C. amphitrete* and the Trematominae from the other clades. The complete mtND2 sequence of *C. amphitrete* differed (unweighted nucleotide differences for single specimens, 1047 nt total length) from that of *P. antarcticum* by 28.4% and from *A. mitopteryx* by 23.5%, but only by 12.8% from *Trematomus newnesi* and by 8.2% from *T. loennbergii*. However, the tree topology within the Trematominae as presented here is not especially well supported by bootstrap values or with additional investigations using mt16S data (not shown) and should be cited with caution. As

suggested by DeWitt et al. (1990), the features shared by *Aethotaxis*, *Gvozdarus*, and *Pleuragramma*, namely, splitting of the supraorbital commissure (or fusion of the fourth supraorbital canal pores), loss of segments in the supratemporal canal (Andersen, 1984), and development of neutral buoyancy, are likely examples of parallel evolution in *Cryothernia*, converging with the Pleuragramminae as it adapted to a more pelagic lifestyle.

It is a surprise that *C. amphitrete* was collected in perhaps the most frequented SCUBA diving and hand-line fishing location in McMurdo Sound, the McMurdo Station saltwater intake jetty. At the time of capture the fish was in excellent condition and did not appear agitated or disoriented. Drifted snow on top of the multi-year ice prevented virtually all sunlight from penetrating to the bottom. The specimen was found resting on a large flat rock (1 m<sup>2</sup>) at approx. 20 m depth; water temperature was -1.91 C with a salinity of 34.7 PSU. The bottom in this area is generally fine volcanic gravel (<30 m) or sponge spicule mat (>30 m) with abundant and diverse benthic megafauna (Dayton et al., 1974; Dayton and Oliver, 1977). The jetty is constructed of large, flat rocks (≥1 m diameter) and attracts many benthic fishes seeking refuge in its caverns and crevices (pers. obs.). Indeed, the Naked Dragonfish, *Gymnodraco acuticeps*, often uses these rare flat surfaces for spawning and depositing its adherent eggs, and as many as 60 individual batches of eggs have been found annually starting in mid-October during the past several field seasons (Evans et al., 2005; pers. obs.). It is possible that the fully gravid *C. amphitrete* found this area equally attractive and was preparing to spawn.

Despite extensive surveys and studies by fellow team-members including Dr. Arthur L. DeVries over the past four decades, to our knowledge *C. amphitrete* has never been previously captured or observed in McMurdo Sound (A. L. DeVries, pers. comm.). This demonstrates that our fishing techniques, limited to divers' hand nets to approx. 30 m, hand-lining, and baited trapping under the annual sea ice are selective and should perhaps be supplemented by remotely operated vehicle (ROV) observations and/or other methods to fully document the McMurdo Sound ichthyofaunal diversity.

*Etymology*.—*Cryothernia* translates from Greek as "from the cold." The name *amphitrete*, literally "an orifice with two openings," describes the interorbital-pit morphology that distinguishes *C. amphitrete* from *C. peninsulae* (Fig. 2).

#### MATERIAL EXAMINED

Comparative morphological examinations used formalin-fixed, ethanol-preserved paratypes of *Cryothernia peninsulae* collected in Peltier Channel (64°52'S, 63°32'W) at 100–200 m depth in 1975 (LACM; 38386.001, 76.5 and 123.9 mm SL, NYSM; 11457, 78.5 and 111.7 mm SL). Phylogenetic investigations (GenBank accession numbers in parentheses; all specimens except *C. amphitrete* without museum voucher) included the mtND2 sequences from the following members of the Nototheniidae: *Aethotaxis mitopteryx* (DQ367402), *C. amphitrete* (holotype, DQ367400; USNM 385901), *Dissostichus mausoni* (AY256561), *Notothenia rossii* (AY256566), *Pagothenia borchgrevinki* (DQ184491), *Pleuragramma antarcticum* (DQ184493), *Trematomus bernacchii* (AY256569), *T. hansonii* (DQ184500), *T. loennbergii* (DQ184502), and *T. newnesi* (DQ184506).

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#### LITERATURE CITED

- ANDERSEN, N. C. 1984. Genera and subfamilies of the family Nototheniidae (Pisces, Perciformes) from the Antarctic and subantarctic. *Steenstrupia* 10:1–34.
- BALUSHKIN, A. V. 1984. Morphological bases of the systematics and phylogeny of the nototheniid fishes. Zoological Institute, Leningrad [In Russian: English translation for the Division of Polar Programs, National Science Foundation, Washington, D.C., by Amerind Publishing Co. Pvt. Ltd., New Delhi, 1989].
- . 2000. Morphology, classification, and evolution of notothenioid fishes of the Southern Ocean (Notothenioidei, Perciformes). *J. Ichthyol.* 40: S74–S109.
- CHENG, C.-H. C., L. CHEN, T. J. NEAR, AND Y. JIN. 2003. Functional antifreeze glycoprotein genes in temperate-water New Zealand nototheniid fish infer an Antarctic evolutionary origin. *Mol. Biol. Evol.* 20:1897–1908.
- CZIKO, P. A., C. W. EVANS, C.-H. C. CHENG, AND A. L. DEVRIES. 2006. Antifreeze proteins and the freezing resistance of larval Antarctic fish. *J. Exp. Biol.* 209:407–420.

- DANIELS, R. A. 1981. *Cryothermia peninsulæ*, a new genus and species of nototheniid fish from the Antarctic Peninsula. *Copeia* 1981:558–562.
- DAYTON, P. K., AND J. S. OLIVER. 1977. Antarctic soft bottom benthos in oligotrophic and eutrophic environments. *Science* 197:55–58.
- , G. A. ROBILIARD, R. T. PAINE, AND L. B. DAYTON. 1974. Biological accommodation in the benthic community at McMurdo Sound, Antarctica. *Ecol. Monogr.* 44:105–128.
- DEVRIES, A. L. 1971. Glycoproteins as biological antifreeze agents in Antarctic fishes. *Science* 172:1152–1155.
- . 1986. Antifreeze glycopeptides and peptides: interactions with ice and water. *Method. Enzymol.* 127:293–303.
- . 1988. The role of antifreeze glycopeptides and peptides in the freezing avoidance of Antarctic fishes. *Comp. Biochem. Physiol.* 90B:611–621.
- DEWITT, H. H., O. GON, AND P. C. HEEMSTRA. 1990. Nototheniidae, p. 279–399. *In: Fishes of the Southern Ocean*. O. Gon and P. C. Heemstra (eds.). J. L. B. Smith Institute of Ichthyology, Grahamstown, South Africa.
- EAKIN, R. R., AND J. T. EASTMAN. 1998. New species of *Pogonophryne* (Pisces, Artedidraconidae) from the Ross Sea, Antarctica. *Copeia* 1998:1005–1009.
- EASTMAN, J. T. 1985. The evolution of neutrally buoyant nototheniid fishes: their specializations and potential interactions in the Antarctic marine food web, p. 430–436. *In: Antarctic Nutrient Cycles and Food Webs*. W. R. Siegfried, P. R. Condy, and R. M. Laws (eds.). Springer-Verlag, Berlin and Heidelberg.
- . 1993. *Antarctic Fish Biology: Evolution in a Unique Environment*. Academic Press, San Diego.
- , AND A. L. DEVRIES. 1981. Buoyancy adaptations in a swimbladderless Antarctic fish *Dissostichus mawsoni*. *J. Morphol.* 167:91–102.
- , AND ———. 1982. Buoyancy studies of nototheniid fishes in McMurdo Sound, Antarctica. *Copeia* 1982:385–393.
- EVANS, C. W., P. A. CZIKO, C.-H. C. CHENG, AND A. L. DEVRIES. 2005. Spawning behavior and early development in the naked dragonfish *Gymnodraco acuticeps*. *Antarct. Sci.* 17:319–327.
- HUBBS, C. L., AND K. F. LAGLER. 1958. *Fishes of the Great Lakes Region*. Cranbrook Institute of Science, Bloomfield Hills, Michigan.
- ILLICK, H. J. 1956. A comparative study of the cephalic lateral-line system of North American Cyprinidae. *Am. Midl. Nat.* 56:204–223.
- JIN, Y., AND A. L. DEVRIES. 2006. Antifreeze glycoprotein levels in Antarctic nototheniid fishes inhabiting different thermal environments and the effect of warm acclimation. *Comp. Biochem. Physiol. B* 144:290–300.
- KOCK, K. H., AND A. KELLERMANN. 1991. Reproduction in Antarctic nototheniid fish. *Antarct. Sci.* 3:125–150.
- KUMAR, S., K. TAMURA, AND M. NEI. 2004. Mega3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief. Bioinform.* 5:150–163.
- POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14:817–818.
- SAMBROOK, J., AND D. W. RUSSELL. 2001. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- WADDELL, P. J., AND M. A. STEEL. 1997. General time-reversible distances with unequal rates across sites: mixing gamma and inverse Gaussian distributions with invariant sites. *Mol. Phylogenet. Evol.* 8:398–414.
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